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PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Remacle, et al.
Appl. No.	:	09/574,626
Filed	:	May 19, 2001
For	:	METHOD FOR THE IDENTIFICATION AND/OR THE QUANTIFICATION OF A TARGET COMPOUND OBTAINED FROM A BIOLOGICAL SAMPLE UPON CHIPS
Examiner	:	Zhou, S.

Group Art Unit 1631

I hereby certify that this correspondence and all marked attachments are being deposited with the United States Postal Service as first-class mail in an envelope addressed to: United States Patent and Trademark Office, P.O. Box 2327, Arlington, VA 22202, on

November 5, 2002
(Date)

Daniel Hart

Daniel Hart, Reg. No. 40,637

DECLARATION UNDER 37 C.F.R §1.132

United States Patent and Trademark Office
P.O. Box 2327
Arlington, VA 22202

Dear Sir:

1. This Declaration is being submitted to demonstrate the significant amplification of signal intensity obtained from methods in which a catalytic reduction forms a metallic precipitate relative to methods which do not employ such a catalytic reduction, such as those described in U.S. Patent No. 6,344,316.

2. I am an inventor on the above-identified patent application and am familiar with the specification and prosecution history.

3. I have extensive experience in the field of biochemistry and cell biology for many years. My Curriculum Vitae has been previously submitted with the Declaration filed on February 22, 2002.

4. A series of slides were prepared as follows:
Slides 9A, 9B, 10 A, 10 B (Upper part) contained capture probes complementary for the fem A gene which is present in different species of Staphylococcus. Each of the capture probe is spotted as triplicate and is specific of the different femA of theses bacterial species. The chips were constructed as explained in the paper of Hamels et al 2001 (Biotechniques 31, 1364-72) obtained from the same inventors. The first upper and lower lines were spotted with biotinylated capture probes and represent positive controls of fixation on the slides. The fem A genes of 5 different bacteria were amplified by consensus primers in the presence of dUTP-biotin and hybridized on the chips. After hybridization and washing they were incubated with blocking buffer (100 mM maleate buffer pH 7.5 ,NaCl 150 mM, Milk powder 0.1%) containing a conjugate antibiotin-gold (17nM dianmeter gold particle) diluted 100X. Incubation was at room temperature for 45 min. Supports were then washed 5X for 1 min in washing buffer composed of 10mM maleate buffer pH7.5 NaCl 15mM Tween 0.1 % and then with water. After the washing, slides were dried and scanned in a black and white scanner similar to dia scanner developed by Eppendorf Array Technology (Namur, belgium). The picture of the digitalized picture of the slide is presentd in the figures. No detection could be observed in these 4 slides by this method where only gold particles are present.

Slides 9A, 9B, 10 A, 10 B (Lower part) and slides 12A and 12B . The slides were the same as here above but a further step was added. The slides were then incubated 8 min in a Silver Blue Solution (AAT, Namur, Belgium), then rinsed in water and dried. The Silver Blue solution is a mixture of silver nitrate and hydroquinone prepared according to the invention and commercialized under the name "Silver Blue" by the company EAT. It allows the reduction of the silver catalysed by the presence of the gold nanoparticles. The slides were scanned in the Black and White scanner as above and the digitalized picture presented in the figure. Clearly, the positive controls of spotting are visible on the lower and upper lines and 5 positive fem A are also visible on all the slides. They correspond to the 5 targets which should be detected. The appearance of signal at locations which were negative was constantly observed after silver precipitation and allow the use of the chips to be analysed with simple black and white scanner making the process particulrly interesting for the users. The genome analysis can be transformed in a black and white analysis of a signal.

5 In addition, as detection of colloidal gold particles without metal precipitation involves measuring the scattering of green light, while silver precipitates such as those utilized in the experiment above are black and may be measured using visible or infra-red light. The inventors also found the Infra red (I-R) gave better signal/noise ratio than visible light. The observation is

probably linked to the fact that silver precipitates are around 800 nM diameter which is close to the wavenlenght of the I-R.thus making the scattering or reflexion maximum. I-R diodes are common and very unexpensive tools and very well adpated for the detectors of the chips according to the invention.

6. As discussed above, utilizing the nanogold particles alone does not yield the sensitivity level and reproducibility level obtained with the metallic precipitates used in the present invention. Observation of slides 10A and 10B, use of the nanogold particle label does not exhibit a signal which gives high sensitivity or reproducibility. The measured signal shown in these two slides (upper slides) shows no signal. In comparison, the 10A and 10B slides after silver precipitation (lower slides) exhibit a high sensitivity and reproducibility, which is further evidenced in slides 12A and 12B. The significant difference in the diameter of the silver precipitates relative to the diameter of the gold particles alone provides an significant signal amplification. If going from 17 to 800 nm particles in diameter represent an increase of volume of 104 000 times. This is probably one of the reason of such sensitivity increase. The inventors also found that they could not immediately use large particles for direct reaction on the chips like for example 800 nm gold particles because the reaction yield and rate were very low or close to zero probably because of the presence of the large and heavy particles. In this invention small gold paricles are used first which react rapidely on the chips and after their specific fixation produce large precipitate trought the silver precipitation process.

7. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 5 November 2002

By: _____
Prof. José REMACLE

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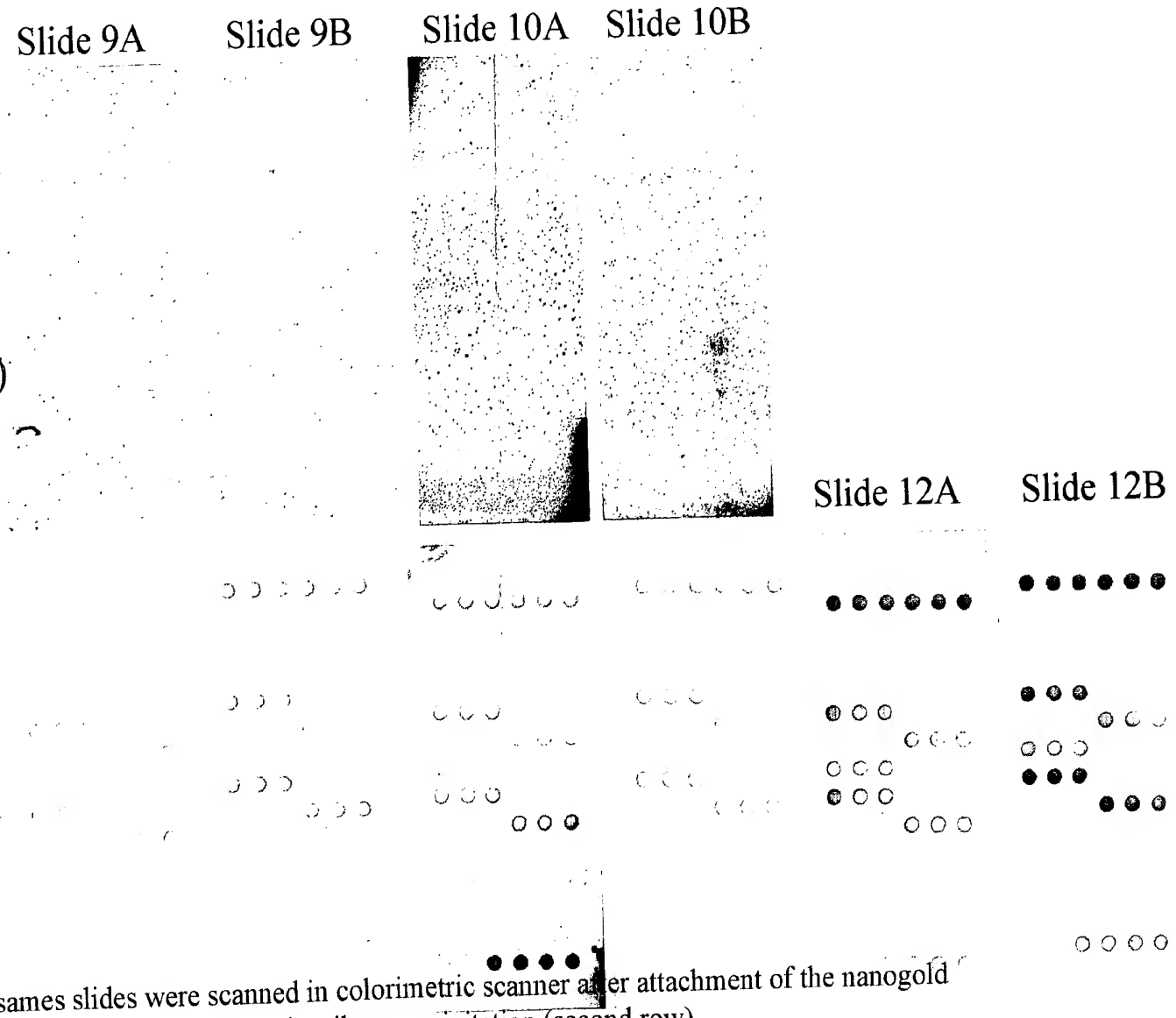
Analysis of the signal before and after the Silver precipitation (08/07/02)



The sames slides were scanned in colorimetric scanner after attachment of the nanogold particles (first row) and after the silver precipitation (second row)